# Chondrocyte fatty acid oxidation drives osteoarthritis via SOX9 degradation and epigenetic regulation

**Zixuan Mei,[1,2, #] Kamuran Yilamu,[1,2, #] Weiyu Ni,[1,2, #] Panyang Shen, [1,2, #] Nan Pan, [1,2] Huasen Chen, [1,2]
Yingfeng Su, [1,2] Lei Guo,[3] Qunan Sun, [4] Zhaomei Li,[5] Dongdong Huang,[3] Xiangqian Fang, [1,2]
Shunwu Fan,[1,2, *] Haitao Zhang,[1,2, *] and Shuying Shen[1,2, *]**

## Inventory of Supporting Information

Figure S1. Lipid accumulation in cartilage accelerates OA progression.
Figure S2. Chondrocytes uptake fatty acids from synovial fluid, contributing to cartilage lipid accumulation.
Figure S3. Acetyl-CoA orchestrates protein acetylation dynamics, rewiring FAO in chondrocytes.
Figure S4. HADHA K728ac is up-regulated under lipid stress and boosts FAO in chondrocytes.
Figure S5. Inhibition of FAO attenuates OA progression *in vitro*.
Figure S6. Inhibition of FAO attenuates OA progression *in vivo*.
Figure S7. FAO inhibited AMPK activity and increased SOX9 degradation.
Figure S8. Phosphorylation of SOX9 impedes its interaction with TRIM9 and enhances its stability.
Figure S9. FFA epigenetically regulates cartilage ECM metabolism.
Figure S10. Isolation of RBC-exosomes (RBC-Exo) and production of CAP-RBC-Exo/TMZ.
Supplementary Table 1. The medical records of patients underwent knee replacement surgery.
Supplementary Table 2. The medical records and laboratory findings from patients underwent synovial fluid collection.
Supplementary Table 3. Oligonucleotide and primer list.
Supplementary Table 4. The OA cartilage histopathology grade.
Supplementary Table 5. The recommended semi-quantitative scoring system (OARSI score).
Supplementary Table 6. The Mankin Histology histopathology grading system.
Supplementary Table 7. Multiple reaction monitoring parameters.
Supplementary Table 8. Key resources table.
Supplementary Data 1. Lipidomic data, related to Figures S1i and S1j.
Supplementary Data 2. Proteomics data, related to Figures S3g and S3h.
Supplementary Data 3. Acetylome data, related to Figures 2c,2d and S3j.
Supplementary Data 4. CUT&Tag-sequencing data, related to Figures 5c,5d,5e and S9b.
Source Data.

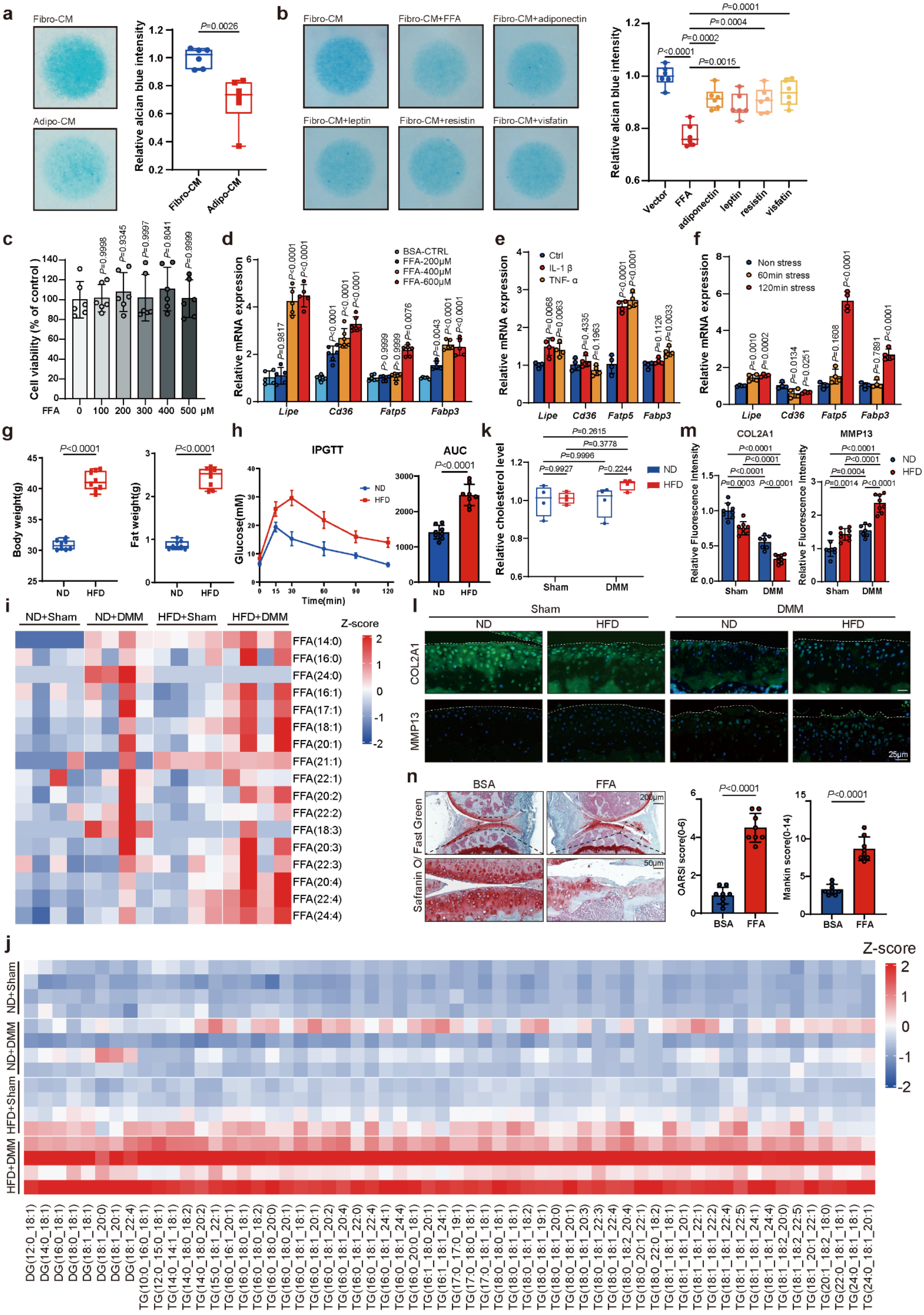
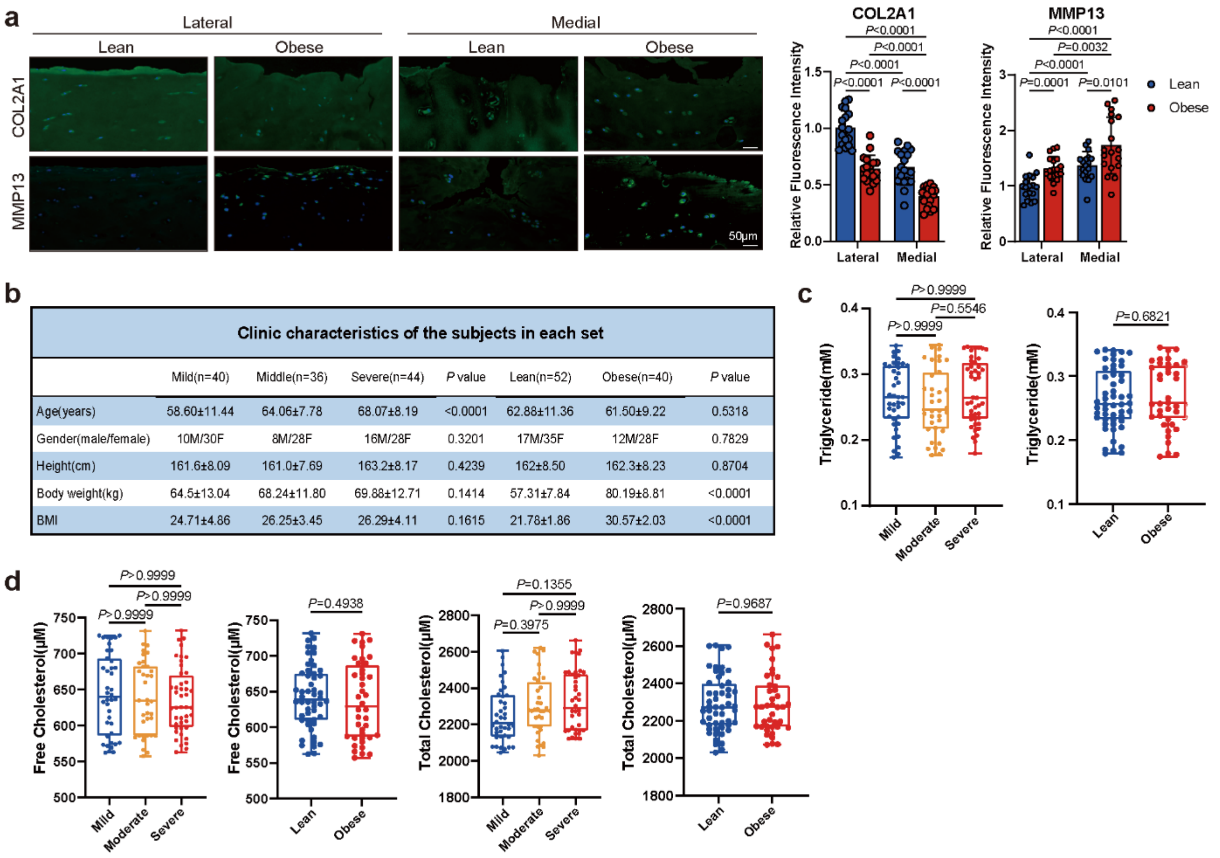


**Figure S1. Lipid accumulation in cartilage accelerates OA progression.** (a-b) Micromass culture and alcian blue staining of primary mouse chondrocytes treated as indicated (n = 6). (c) Cell viability of mouse chondrocytes treated with specific concentrations of FFA (n=6). (d) Changes in *Lipe*, *Cd36*, *Fatp5*, and *Fabp3* mRNA levels in primary murine chondrocytes, treated with FFA, as determined via RT-qPCR analysis (n= 5 for *Lipe and Fabp3*; n= 6 for *Cd36* and *Fatp5*). (e) Changes in *Lipe*, *Cd36*, *Fatp5*, and *Fabp3* mRNA levels in primary murine chondrocytes, treated with IL-1β and TNF-α, as determined via RT-qPCR analysis (n = 4). (f) Changes in *Lipe*, *Cd36*, *Fatp5*, and *Fabp3* mRNA levels in primary murine chondrocytes treated with tensile-stress loading, as determined via RT-qPCR analysis (n = 4). (g) Body weight and inguinal adipose tissue weight of mice indicated in Figure 1f before sacrifice (n = 8). (h) Intraperitoneal glucose tolerance test (IPGTT) results before sacrifice (n = 8). (i) Heatmap showing the levels of FFAs with different lengths in cartilage samples from mice indicated in Figure 1f. (j) Heatmap showing the top 60 upregulated GL species containing FFAs with different lengths in cartilage samples from mice as indicated in Figure 1f. (k) Relative cholesterol level in cartilage samples from mice indicated in Figure 1f (n = 4). (l-m) IF staining for COL2A1 and MMP13 in mouse knee joints indicated in Figure 1i (n = 7 for ND DMM group; n = 8 for ND Sham, HFD Sham, and HFD DMM groups). (n) Safranin O/Fast green staining of knee joints from mice treated with FFA or BSA arthrocentesis (n = 8). (a, b, g, and k) Data are minimum to maximum: each point represents an individual biological replication, box from 25th to 75th percentiles; center line at median; tukey whiskers, as determined via unpaired two-tailed *t*-test (a, b, and g) or two-way ANOVA followed by tukey's multiple-comparisons test (k). (c-f, h, and m-n) Data are mean ±SD, as determined by one-way ANOVA followed by Dunn's multiple-comparisons test (c, f) or two-tailed *t*-test or nonparametric two-tailed Mann–Whitney test (e, h, and m-n). Source data are provided as a Source Data file.



Clinic characteristics of the subjects in each set

| | Mild(n=40) | Middle(n=36) | Severe(n=44) | *P* value | Lean(n=52) | Obese(n=40) | *P* value |
|---|---|---|---|---|---|---|---|
| Age(years) | 58.60±11.44 | 64.06±7.78 | 68.07±8.19 | <0.0001 | 62.88±11.36 | 61.50±9.22 | 0.5318 |
| Gender(male/female) | 10M/30F | 8M/28F | 16M/28F | 0.3201 | 17M/35F | 12M/28F | 0.7829 |
| Height(cm) | 161.6±8.09 | 161.0±7.69 | 163.2±8.17 | 0.4239 | 162±8.50 | 162.3±8.23 | 0.8704 |
| Body weight(kg) | 64.5±13.04 | 68.24±11.80 | 69.88±12.71 | 0.1414 | 57.31±7.84 | 80.19±8.81 | <0.0001 |
| BMI | 24.71±4.86 | 26.25±3.45 | 26.29±4.11 | 0.1615 | 21.78±1.86 | 30.57±2.03 | <0.0001 |

**Figure S2. Chondrocytes uptake fatty acids from synovial fluid, contributing to cartilage lipid accumulation.** (a) IF staining for COL2A1 and MMP13 in human knee cartilage tissues indicated in Figure 1j (n = 18). (b) Clinical data of patients, including age, gender, height, body weight, and BMI. (c) The level of total triglycerides in synovial fluids from patients with mild (n = 40), moderate (n = 36), and severe (n = 44) OA (Left), and from patients classified as Lean (n = 52) or with obesity (n = 40) (Right). (d) The levels of free cholesterols and total cholesterols in synovial fluids from patients with mild (n = 40), moderate (n = 36), and severe (n = 44) OA (Left), and from patients classified as Lean (n = 52) or with obesity (n = 40) (Right). (a) Data are mean ±SD, as determined by two-tailed *t*-test or nonparametric two-tailed Mann–Whitney test. (c-d) Data are minimum to maximum: each point represents an individual biological replication, box from 25th to 75th percentiles; center line at median; tukey whiskers, as determined via nonparametric Kruskal–Wallis test followed by Dunn's multiple comparisons test, or nonparametric two-tailed Mann–Whitney test. Source data are provided as a Source Data file.

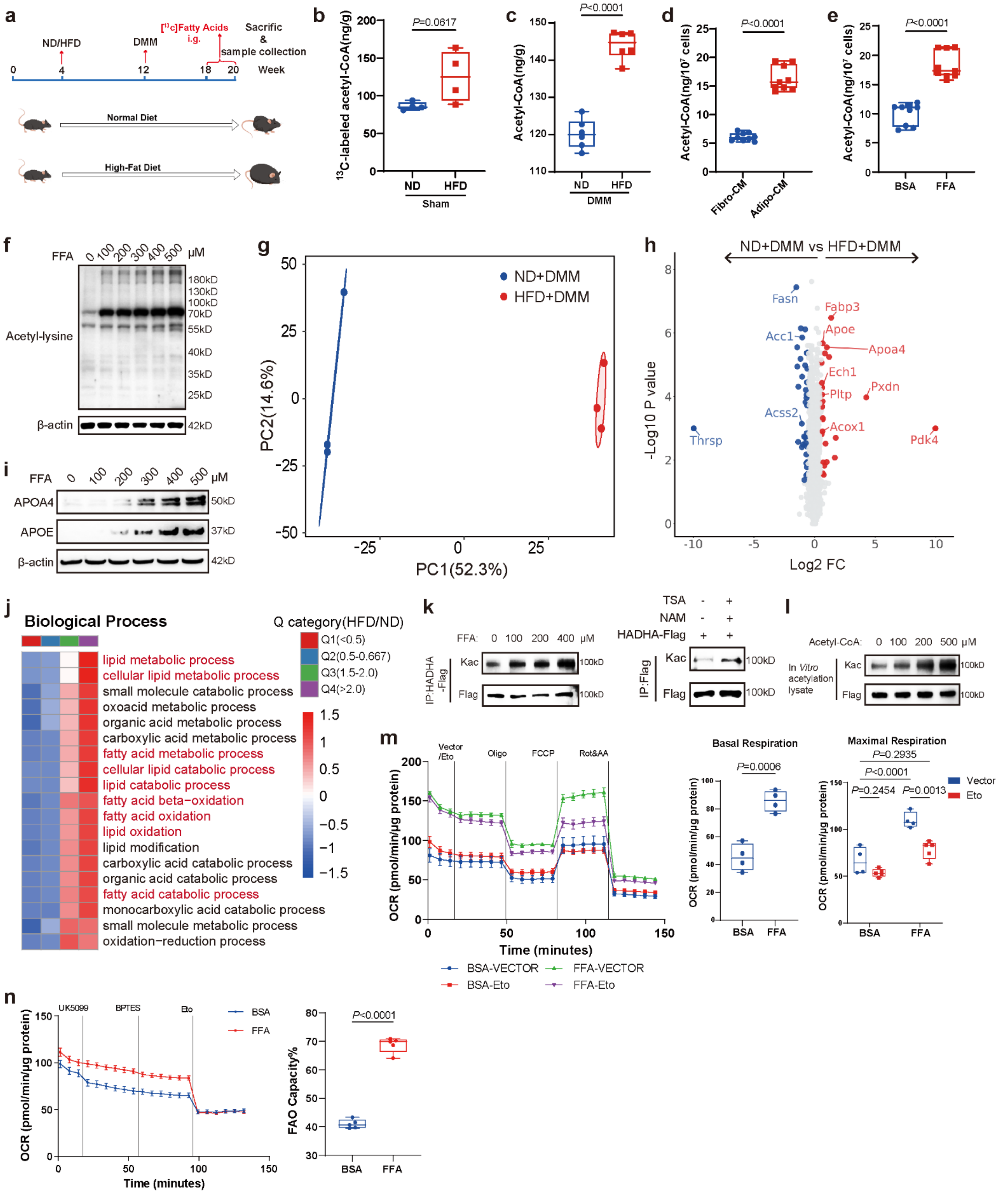


**Figure S3. Acetyl-CoA orchestrates protein acetylation dynamics, rewiring FAO in chondrocytes.** (a) Illustration of diet change and destabilization of the DMM surgery or sham operation in male C57BL/6J mice, two weeks before sacrifice; all mice were orally gavaged with [$^{13}$C] fatty acids every day, by figdraw.com. (b) Quantification of $^{13}$C-labeled acetyl-CoA levels in cartilage samples from mice that underwent Sham operation, as indicated above (n = 4). Data represent the minimum to maximum; each point represents an individual biological replicate, as determined via nonparametric two-tailed Mann–Whitney test. (c-e) The level of acetyl-CoA in cartilage samples from ND+DMM and HFD+DMM mice (c, n = 6) and in primary murine chondrocytes treated with adipo-CM or fibro-CM (d, n = 9), FFA, or BSA (e, n = 9), assessed via ELISA. Data represent the minimum to maximum; each point represents an individual biological replicate, as determined via unpaired two-tailed *t*-test. (f) Western blot analysis of acetyl-lysine from whole cell lysate harvested from primary murine chondrocytes treated with FFA. (g) PCA analysis for visualizing the protein alterations among experimental groups. (h) Volcano plot analysis of proteins altered in HFD+DMM versus ND+DMM groups, via two-tailed t-test. (i) Immunoblot detection of APOA4 and APOE in primary mouse chondrocytes treated with FFA. (j) GO enrichment analysis of acetylation sites showing enriched BP terms in HFD+DMM versus ND+DMM groups ($P < 0.05$), via two-tailed Fisher's exact test. (k-l) Immunoblots showing HADHA acetylation when overexpressed in C28/I2 cells treated as indicated or *in vitro* acetylation in lysates containing indicated concentrations of acetyl-CoA. (m) Extracellular-flux analysis (EFA) of OCRs. Basal respiration and maximal respiration were calculated using Wave 2.4.0 ($n = 4$ for Vector treated groups; $n = 5$ for Eto treated groups). Data are mean ±SEM or minimum to maximum: each point represents an individual biological replicate, as determined via unpaired two-tailed t-test or two-way ANOVA followed by Tukey's multiple-comparisons test. (n) Mitochondrial fuel oxidation analysis ($n = 5$). Data are mean ±SEM or minimum to maximum: each point represents an individual biological replicate, as determined via unpaired two-tailed t-test. The western blot data are representative of three independent experiments. Source data are provided as a Source Data file.

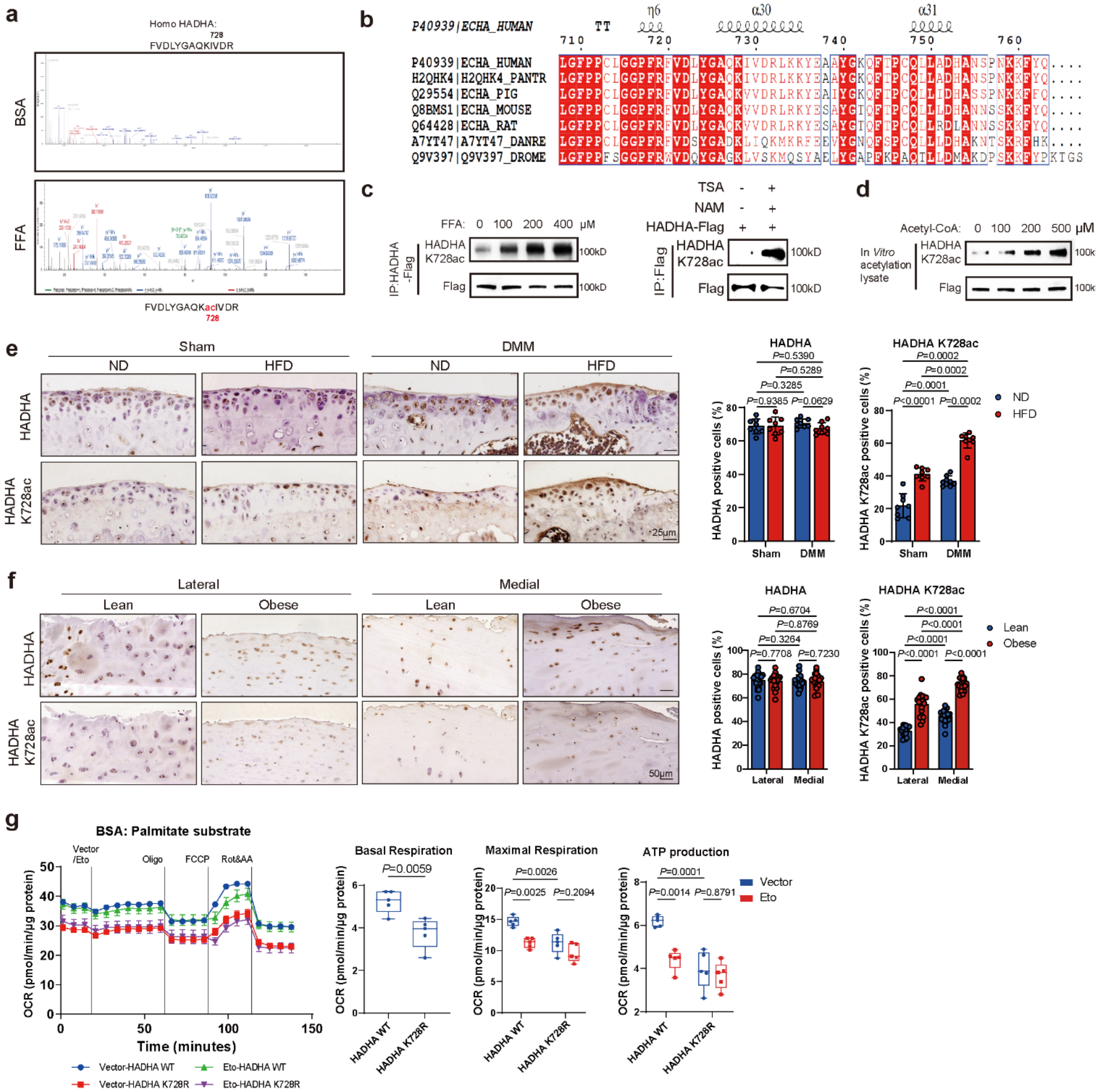


**Figure S4. HADHA K728ac is up-regulated under lipid stress and boosts FAO in chondrocytes.** (a) UPLC-MS/MS for identifying the acetylation site on HADHA in C28/I2 cells treated with FFA or BSA. (b) Structure-based sequence alignments of HADHA amino acids 708 to 763, carried out using ESPript 3.0. (c-d) Immunoblot showing HADHA K728 acetylation when overexpressed in C28/I2 cells treated as indicated or *in vitro* acetylation in lysates containing indicated concentrations of acetyl-CoA. (e) IHC staining for HADHA and HADHA K728ac in mouse knee joints indicated in Figure 1h (n = 8). Data represent the mean± SD, as determined via two-tailed *t*-test or nonparametric two-tailed Mann–Whitney test. (f) IHC staining for HADHA and HADHA K728ac in human knee cartilage indicated in Figure 1j (n = 18 for Lean group; n = 20 for Obese group). Data represent the mean± SD, as determined via two-tailed *t*-test or nonparametric two-tailed Mann–Whitney test. (g) EFA of FAO substrate OCRs. After 24 h of starvation, HADHA-KO C28/I2 cells overexpressing wild-type HADHA or HADHA K728R were treated with BSA-conjugated palmitate 15 min before loading. Basal respiration, maximal respiration, and ATP production were calculated using Wave 2.4.0 (n = 5). Data are mean ±SEM or minimum to maximum: each point represents an individual biological replicate, as determined via unpaired two-tailed t-test or two-way ANOVA followed by Tukey's multiple-comparisons test. The western blot data are representative of three independent experiments. Source data are provided as a Source Data file.

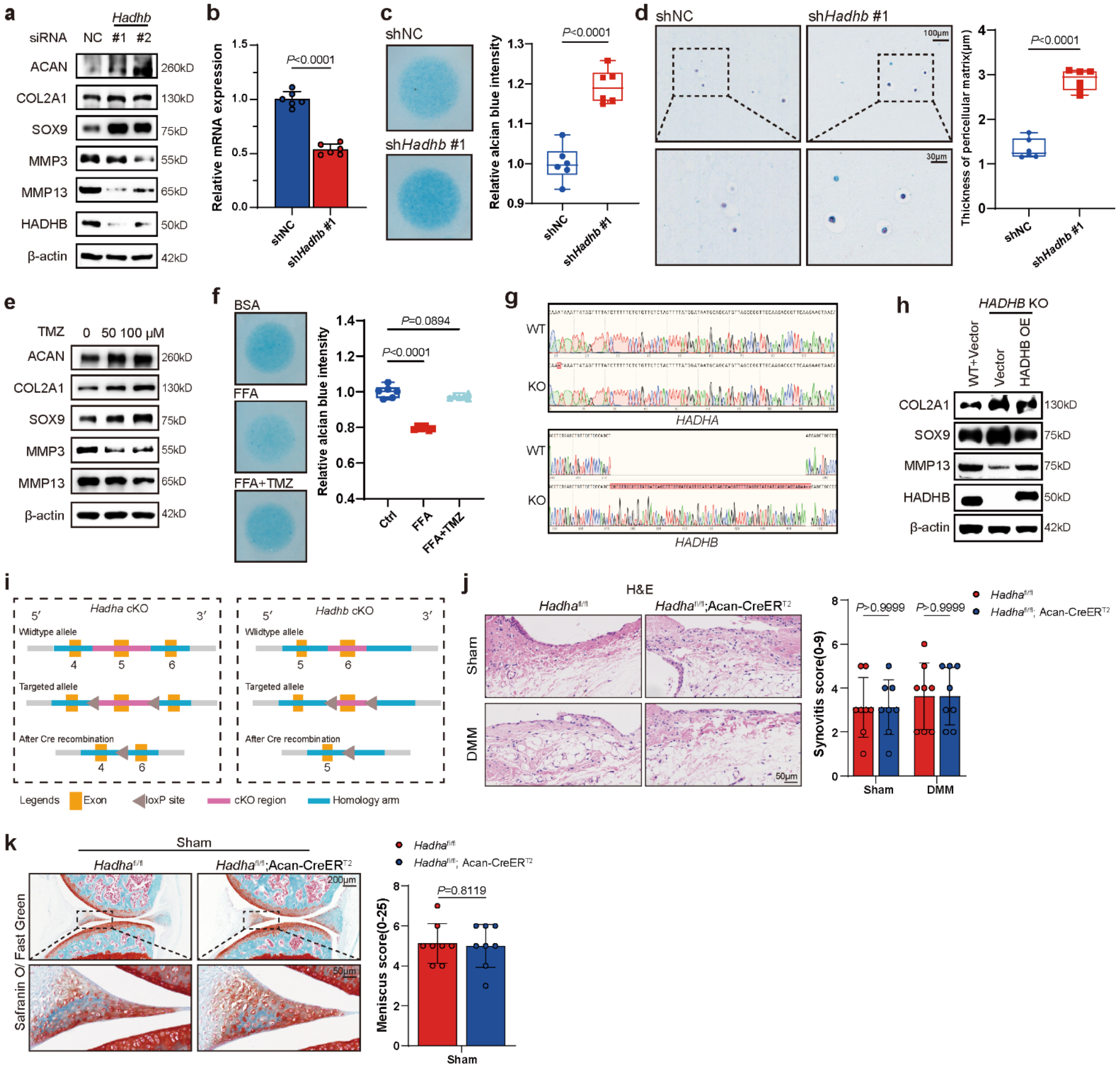


**Figure S5. Inhibition of FAO attenuates OA progression *in vitro*.** (a) Immunoblot detection of ACAN, COL2A1, SOX9, MMP3, MMP13, and HADHB in mouse chondrocytes transfected with *Hadhb* siRNAs. All cells were treated with 200 μM FFA. (b) mRNA expression levels of *Hadha* in mouse chondrocytes infected with a lentivirus encoding *Hadhb* short hairpin RNA #1 (n = 6). (c-d) Micromass culture(c) or 3D-agarose culture (d), along with alcian blue staining of murine chondrocytes infected with a lentivirus encoding *Hadhb* short hairpin RNA #1 (n = 6). Notably, the images and statistical data of shNC were incorporated into Figure 3c-3d as the experiments were performed simultaneously with a single control experiment. (e) Immunoblot detection of ACAN, COL2A1, SOX9, MMP3, and MMP13 in TMZ-treated mouse chondrocytes. All cells were treated with 200 μM FFA. (f) Micromass culture and alcian blue staining of murine chondrocytes treated with FFA ± TMZ (n = 6). (g) Illustrates the DNA sequence surrounding the sgRNA targeting regions in *HADHA*-KO and *HADHB*-KO C28/I2 cells. (h) Immunoblot detection of COL2A1, SOX9, MMP13, and HADHB in *HADHB*-KO C28/I2 cells overexpressing vector or HADHB. All cells were treated with 200 μM FFA. (i) Gene editing strategy for *Hadha* and *Hadhb* conditional knock-out mouse. (j) Hematoxylin and eosin staining (H&E) of synovial tissues from knee joints of *Hadha*$^{fl/fl}$ and *Hadha*$^{fl/fl}$; Acan-creER$^{T2}$ mice. Synovitis scores was used to assess histological changes in the synovial tissues (n = 8). (k) Safranin O/Fast green staining for HADHA in knee joints of *Hadha*$^{fl/fl}$ and *Hadha*$^{fl/fl}$; Acan-creER$^{T2}$ mice that underwent sham surgery. Meniscus scores was used to assess histological changes in the meniscus (n = 8). (b, and j-k) Data are mean ± SD, as determined via unpaired two-tailed *t*-test or nonparametric two-tailed Mann–Whitney test. (c-f) Data are minimum to maximum: each point represents an individual biological replicate, box from 25th to 75th percentiles; center line at median; tukey whiskers, as determined via unpaired two-tailed t-test (c-d) or one-way ANOVA followed by Tukey’s multiple-comparisons test (f). The immunoblotting data are representative of three independent experiments. Source data are provided as a Source Data file.

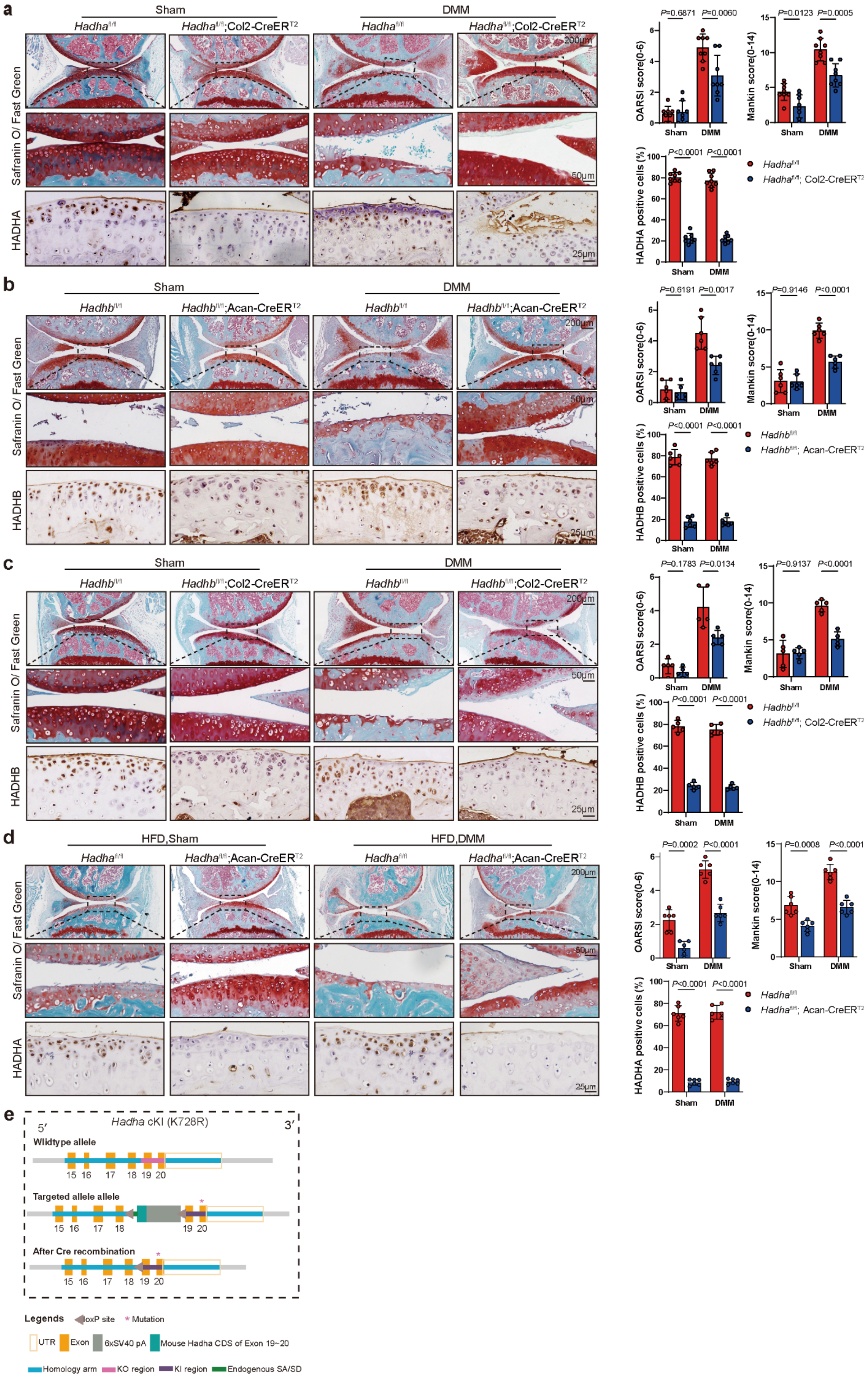


**Figure S6. Inhibition of FAO attenuates OA progression *in vivo*.** (a) Safranin O/Fast green staining and IHC staining for HADHA in knee joints of *Hadha*$^{fl/fl}$ and *Hadha*$^{fl/fl}$; Col2-creER$^{T2}$ mice that underwent DMM or sham surgery. Samples were collected 12 weeks after surgery (n = 8). (b) Safranin O/Fast green staining and IHC staining for HADHB in knee joints of *Hadhb*$^{fl/fl}$ and *Hadhb*$^{fl/fl}$; Acan-creER$^{T2}$ mice that underwent DMM or sham surgery. Samples were collected 12 weeks after surgery (n = 6). (c) Safranin O/Fast green staining and IHC staining for HADHB in knee joints of *Hadhb*$^{fl/fl}$ and *Hadhb*$^{fl/fl}$; Col2-creER$^{T2}$ mice that underwent DMM or sham surgery. Samples were collected 12 weeks after surgery (n = 5). (d) Safranin O/Fast green staining and IHC staining for HADHA in knee joints of *Hadha*$^{fl/fl}$ and *Hadha*$^{fl/fl}$; Acan-creER$^{T2}$ mice that underwent DMM or sham surgery at 12 weeks of age. Mice were started on an HFD at 4 weeks, with samples collected 8 weeks after surgery (n = 8). (e) Gene editing strategy for HADHA conditional knock-in (K728R) mouse. Data are mean ± SD, as determined via unpaired two-tailed *t*-test or nonparametric two-tailed Mann–Whitney test. Source data are provided as a Source Data file.

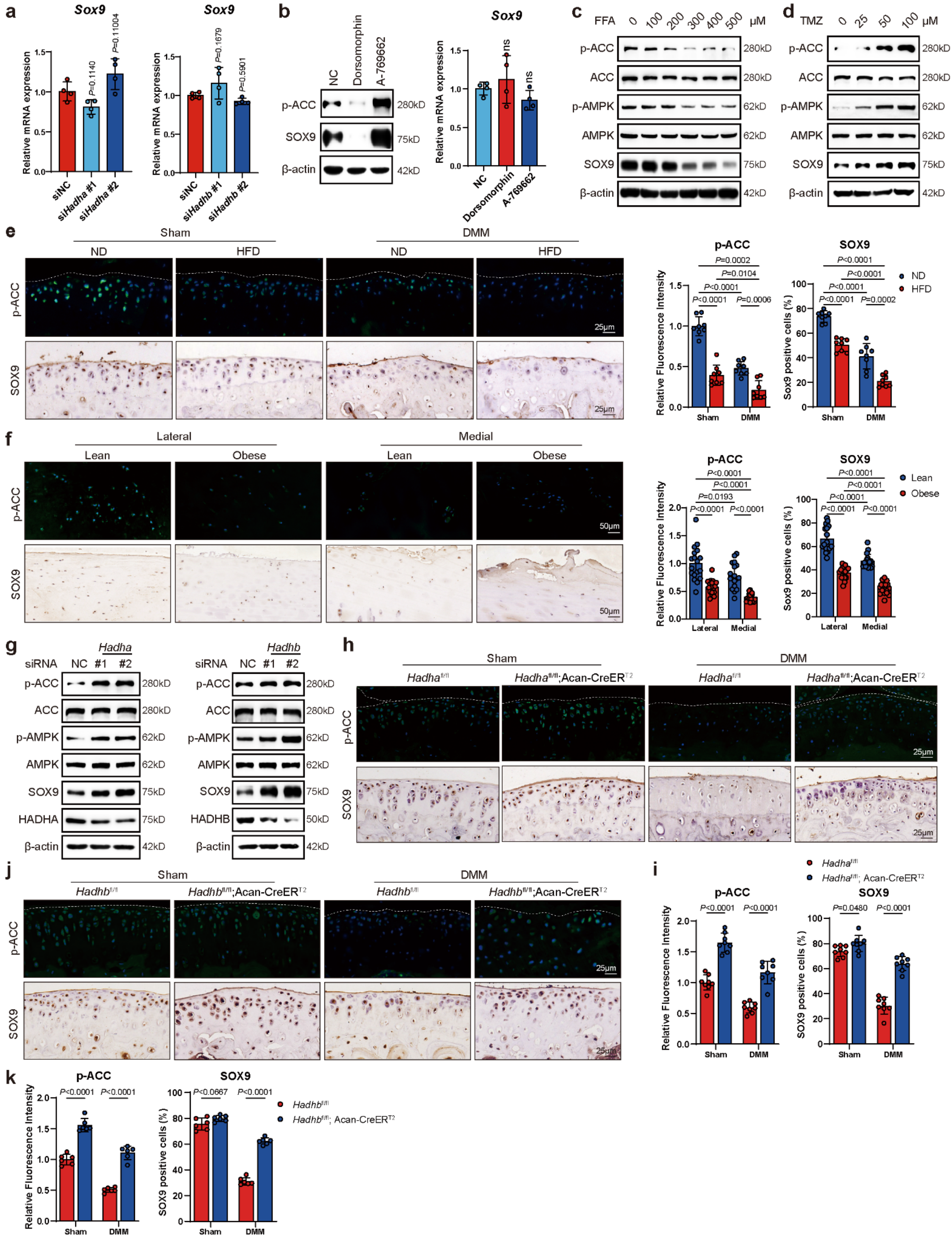


**Figure S7. FAO inhibited AMPK activity and increased SOX9 degradation.** (a) *Sox9* mRNA levels in mouse chondrocytes transfected with *Hadha* and *Hadhb* siRNAs. (b) Immunoblot of p-ACC and SOX9, and *Sox9* mRNA level in primary mouse chondrocytes treated with dorsomorphin and A-769662 overnight (n = 4). (c-d) Immunoblot of p-ACC/ACC, p-AMPK/AMPK, and SOX9 in primary murine chondrocytes with indicated treatment. (e) IF staining for p-ACC and IHC staining for SOX9 in mouse knee cartilage shown in Figure 1h (n = 8). (f) IF staining for p-ACC and IHC staining for SOX9 in human knee cartilage shown in Figure 1j (n = 18). (g) Immunoblot of p-ACC/ACC, p-AMPK/AMPK, and SOX9 in primary mouse chondrocytes transfected with *Hadha* and *Hadhb* siRNAs. (h-i) IF staining for p-ACC and IHC staining for SOX9 in mouse knee cartilage indicated in Figure 3h (n = 8). (j-k) IF staining for p-ACC and IHC staining for SOX9 in mouse knee cartilage shown in Figure S6b (n = 6). Data represent the mean ± SD, as determined by one-way ANOVA, test followed by Dunnett's multiple comparisons test (a, b) or unpaired two-tailed t-test or nonparametric two-tailed Mann–Whitney test (e-k). The western blot data are representative of three independent experiments. Source data are provided as a Source Data file.

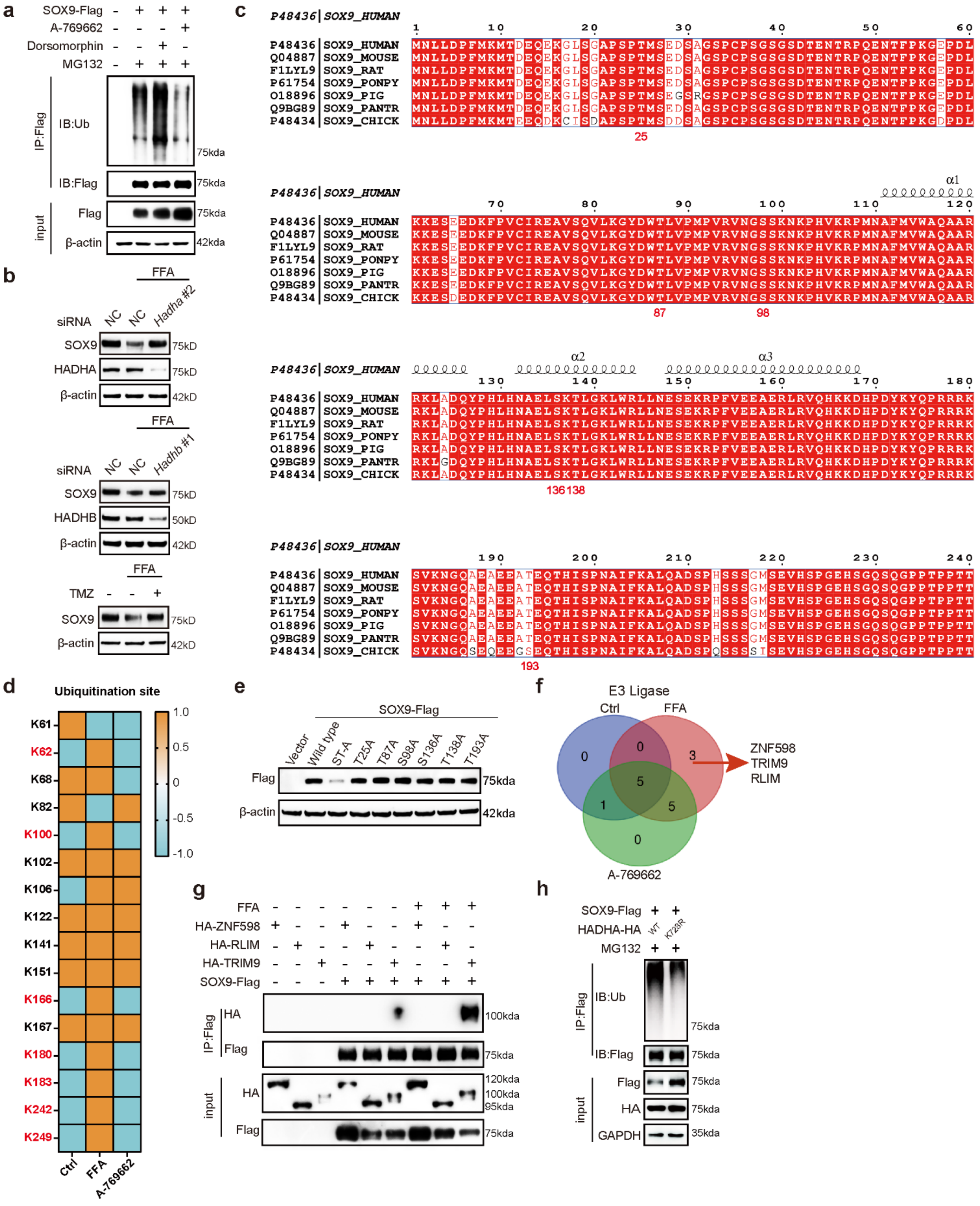


**Figure S8. Phosphorylation of SOX9 impedes its interaction with TRIM9 and enhances its stability.** (a) Immunoblot detection of ubiquitinated Flag-tagged SOX9 overexpressed in C28/I2 cells via Flag immunoprecipitation. The cells were treated as indicated for 12h and with MG132 for 6 h before harvesting. (b) Immunoblot detection of SOX9 in mouse chondrocytes treated as indicated. (c) Structure-based amino-acid sequence alignments of SOX9 (amino acids 1 to 240 are shown) carried out using ESPript 3.0. (d) Heatmap showing the results of UPLC-MS/MS analysis for identifying ubiquitination sites on the SOX9 protein purified from C28/I2 cells. The presence, absence, or lack of detection of modifications at the specified sites in the SOX9 protein are denoted by '1', '–1', and '0' respectively. (e) Immunoblot analysis of Flag tagged-wild-type or mutated SOX9 in HEK293T cells. The cells were pretreated with CHX for 3 hours. The basal SOX9 protein levels were adjusted to equivalent amounts by controlling the plasmid transfection dosage. (f) Venn diagram showing that the E3 ubiquitin ligase interacted with SOX9 purified from C28/I2 cells treated as indicated. (g) Co-immunoprecipitation of Flag-tagged SOX9 with HA-tagged ZNF598, RLIM, and TRIM9 in HEK293T cells treated with FFA or BSA. (h) Immunoblot detection of ubiquitinated SOX9, isolated from *HADHA*-KO C28/I2 cells overexpressing wild-type HADHA or HADHA K728R via anti-Flag tag immunoprecipitation. The western blot data are representative of three independent experiments. Source data are provided as a Source Data file.

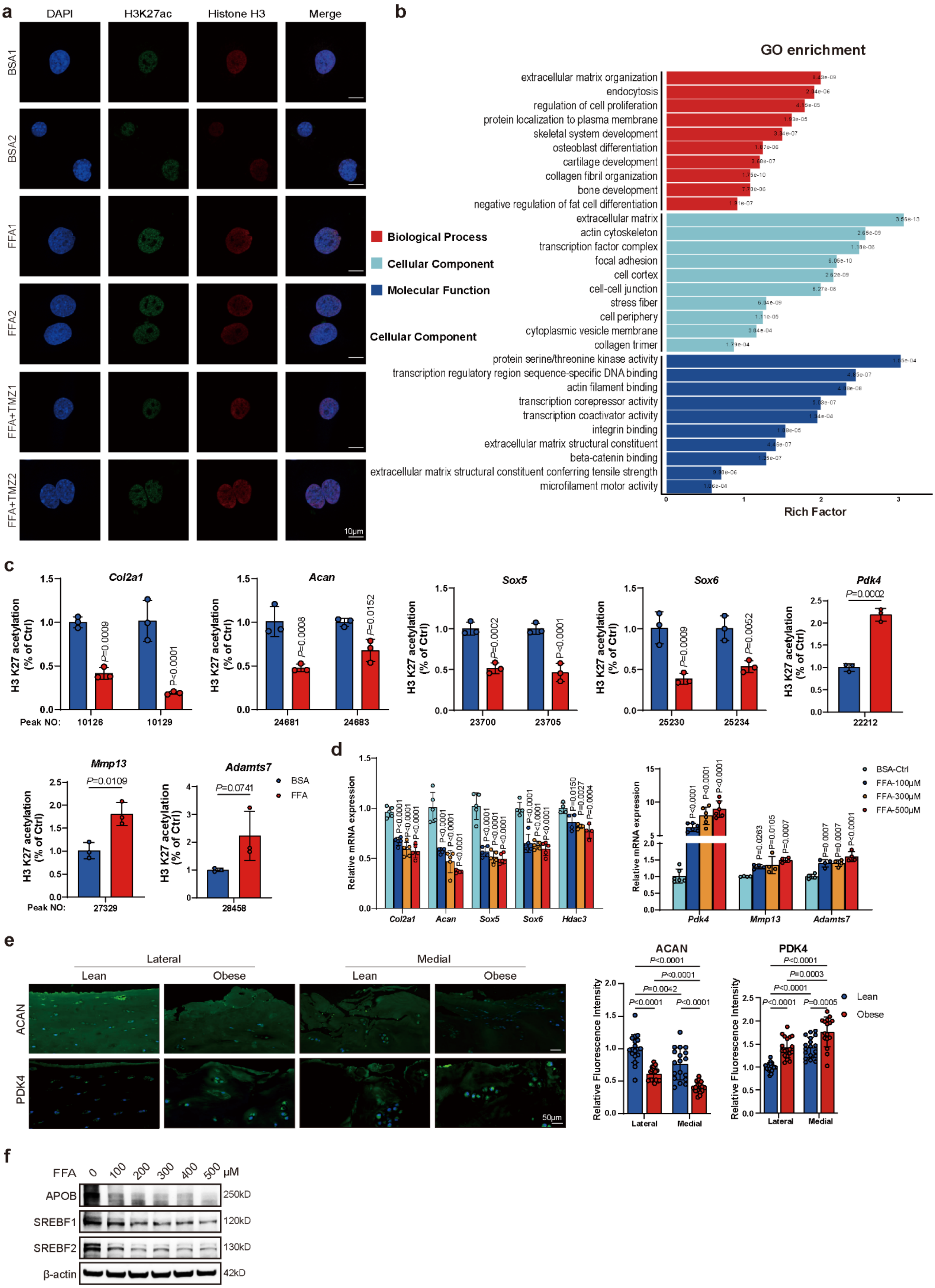


**Figure S9. FFA epigenetically regulates cartilage ECM metabolism.** (a) Representative confocal images of IF staining for H3K27ac and histone H3 in primary mouse chondrocytes treated with BSA, FFA, or FFA + TMZ. (b)

GO enrichment analysis of genes around significantly altered H3K27ac peaks ($\log_2$ FC > 1, $P < 0.01$), as determined by a two-tailed Fisher's exact test followed by Benjamini–Hochberg (BH) correction. (c) Quantification of H3K27 acetylation at the gene locus, including *Col2a1*, *Acan*, *Sox5*, *Sox6*, *Pdk4*, *Mmp13*, and *Adamts7*, in BSA- and FFA-treated chondrocytes, assessed via CUT&Tag-qPCR (n = 3). Data are mean ± SD, based on unpaired two-tailed *t*-test. (d) mRNA expression levels of *Col2a1* (n = 5), *Acan* (n = 5), *Sox5* (n = 5), *Sox6* (n = 5), *Hdac3* (n = 4), *Pdk4* (n = 6), *Mmp13* (n = 4), and *Adamts7* (n = 4) in mouse chondrocytes treated with the indicated concentrations of FFA, as assessed via RT-qPCR. Data are mean ±SD, based on one-way ANOVA followed by Dunnett's multiple-comparisons test. (e) IF staining for ACAN and PDK4 in human knee cartilage indicated in Figure 1k (n = 18). Data are mean ±SD, based on two-tailed *t*-test or nonparametric two-tailed Mann–Whitney test. (f) Immunoblot detection of APOB, SREBF1 and SREBF2 in primary mouse chondrocytes treated with FFA. The western blot data are representative of three independent experiments. Source data are provided as a Source Data file.

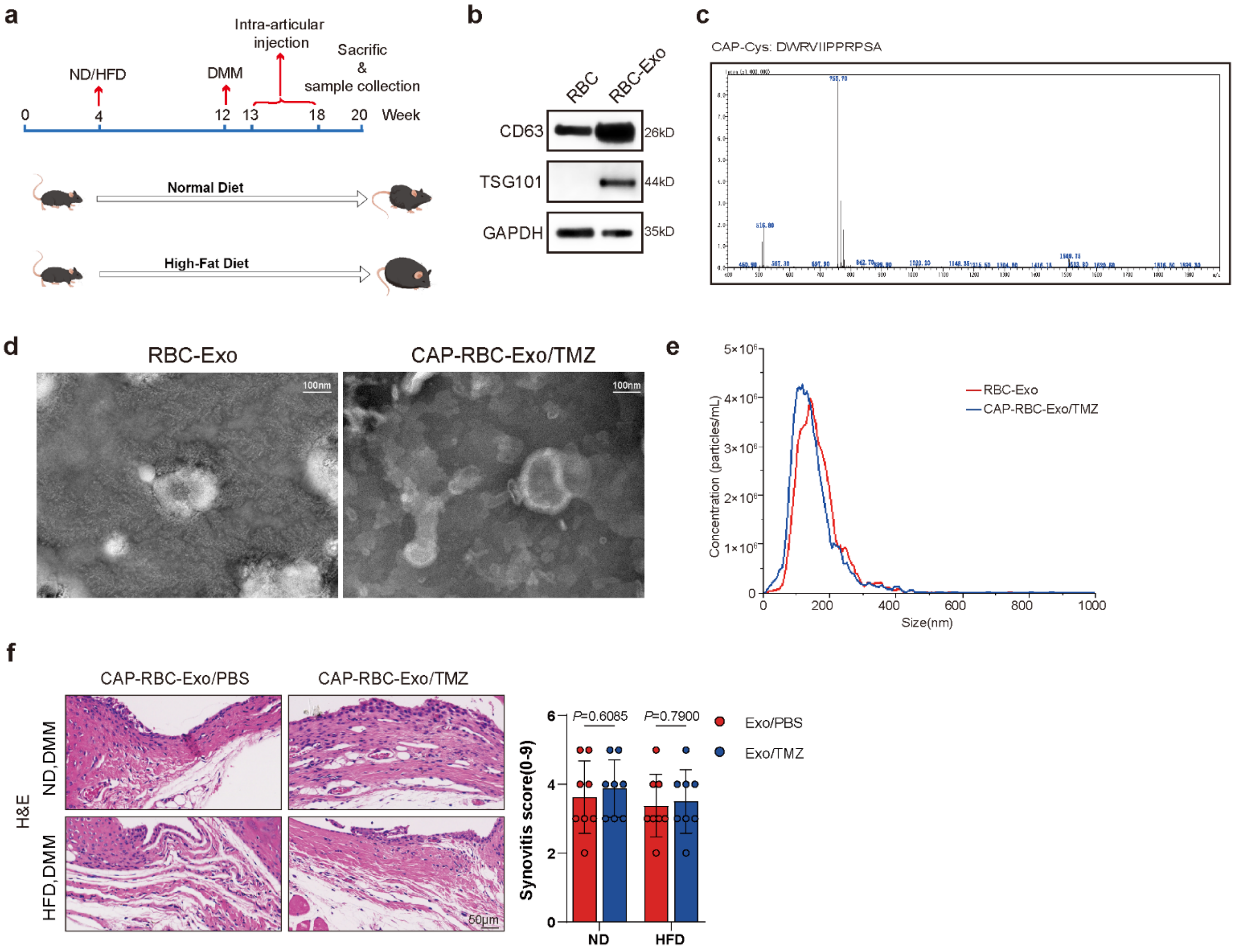


**Figure S10. Isolation of RBC-exosomes (RBC-Exo) and production of CAP-RBC-Exo/TMZ.** (a) Illustration of diet change and DMM surgery and arthrocentesis in male C57BL/6J mice, by figdraw.com. (b) Expression of marker proteins in whole cell lysates and lysates of purified exosomes examined by western blotting. (c) UPLC-MS/MS for identifying the chondrocyte-affinity peptide (CAP). (d) Representative transmission electron microscopy images of RBC-Exo and CAP-RBC-Exo/TMZ. (e) Size distribution of RBC-Exo and CAP-RBC-Exo/TMZ, determined by nanoparticle tracking analysis. (f) Hematoxylin and eosin staining (H&E) of synovial tissues in knee joints form mice indicated in Figure 6b. Synovitis scores was used to assess histological changes in the synovial tissues (n = 8). The data represent the mean ± SD, as determined by two-tailed *t*-test or nonparametric two-tailed Mann–Whitney test. Source data are provided as a Source Data file.

**Supplementary Table 1. The medical records of patients underwent knee replacement surgery.**

| No. | Gender | Age(years) | Height(cm) | Body Weight(kg) | Body Mass Index | Kellgren &Lawrence Grade |
|---|---|---|---|---|---|---|
| 1 | Female | 76 | 158 | 52 | 20.82 | IV |
| 2 | Female | 74 | 160 | 60 | 23.44 | IV |
| 3 | Female | 75 | 160 | 60 | 23.44 | IV |
| 4 | Male | 66 | 175 | 73 | 23.84 | Ⅲ |
| 5 | Female | 54 | 150 | 52 | 23.11 | IV |
| 6 | Male | 68 | 155 | 58 | 23.73 | IV |
| 7 | Female | 60 | 163 | 60 | 22.58 | Ⅲ |
| 8 | Female | 71 | 160 | 61 | 23.83 | IV |
| 9 | Female | 63 | 155 | 57 | 23.73 | IV |
| 10 | Female | 70 | 160 | 60 | 23.44 | IV |
| 11 | Female | 67 | 165 | 62 | 22.78 | Ⅱ |
| 12 | Female | 59 | 165 | 55 | 20.20 | Ⅲ |
| 13 | Female | 76 | 155 | 57 | 23.73 | Ⅲ |
| 14 | Female | 71 | 158 | 59 | 23.63 | IV |
| 15 | Female | 67 | 153 | 56 | 23.92 | IV |
| 16 | Female | 76 | 155 | 48 | 19.98 | IV |
| 17 | Female | 54 | 160 | 60 | 23.44 | IV |
| 18 | Female | 74 | 160 | 61 | 23.83 | IV |
| 19 | Female | 73 | 165 | 87 | 31.96 | Ⅲ |
| 20 | Female | 63 | 162 | 63 | 28.32 | Ⅲ |
| 21 | Female | 63 | 160 | 89 | 34.77 | Left:IV Right:IV |
| 22 | Male | 75 | 173 | 87 | 29.07 | Ⅲ |

| | | | | | | |
|---|---|---|---|---|---|---|
| 23 | Male | 76 | 160 | 80 | 31.25 | IV |
| 24 | Female | 64 | 160 | 76 | 29.69 | IV |
| 25 | Female | 73 | 146 | 62 | 29.09 | IV |
| 26 | Female | 62 | 160 | 85 | 33.20 | IV |
| 27 | Female | 80 | 155 | 72 | 29.97 | IV |
| 28 | Female | 77 | 150 | 65 | 28.89 | IV |
| 29 | Male | 64 | 170 | 82 | 28.37 | IV |
| 30 | Female | 60 | 156 | 69 | 28.35 | Left:IV Right:IV |
| 31 | Female | 82 | 160 | 72 | 28.13 | IV |
| 32 | Female | 64 | 160 | 72 | 28.13 | IV |
| 33 | Female | 73 | 153 | 70 | 29.90 | Left:Ⅲ Right:IV |
| 34 | Female | 64 | 171 | 82 | 28.04 | IV |
| 35 | Female | 58 | 165 | 85 | 31.22 | Left:IV Right:Ⅲ |
| 36 | Female | 59 | 162 | 85 | 32.39 | IV |

**Supplementary Table 2. The medical records and laboratory findings from patients underwent synovial fluid collection.**

| NO. | Age (years) | Gender | Height (cm) | Body weight (kg) | BMI | K & L Grade | Synovial fluid FFA (μM) | Synovial fluid TG (mM) | Synovial fluid FC (μM) | Synovial fluid TC (μM) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 62 | Female | 152 | 45 | 19.48 | 0 | 575.58 | 0.27 | 721.68 | 2291.26 |
| 2 | 31 | Male | 175 | 65 | 21.22 | 0 | 721.90 | 0.32 | 724.82 | 2256.71 |
| 3 | 50 | Female | 158 | 53 | 21.23 | 0 | 597.87 | 0.29 | 642.53 | 2046.56 |
| 4 | 52 | Female | 164 | 58 | 21.56 | 0 | 589.15 | 0.23 | 619.77 | 2254.16 |
| 5 | 64 | Male | 166 | 60 | 21.77 | 0 | 440.89 | 0.18 | 693.48 | 2467.98 |
| 6 | 27 | Male | 177 | 69 | 22.02 | 0 | 331.40 | 0.27 | 672.21 | 2214.63 |
| 7 | 52 | Female | 160 | 57 | 22.27 | 0 | 370.16 | 0.32 | 636.26 | 2187.60 |
| 8 | 65 | Female | 165 | 65 | 23.88 | 0 | 597.87 | 0.31 | 649.62 | 2198.93 |
| 9 | 45 | Female | 157 | 68 | 27.59 | 0 | 395.35 | 0.34 | 568.98 | 2065.82 |
| 10 | 53 | Female | 153 | 68 | 29.05 | 0 | 739.34 | 0.25 | 689.53 | 2274.83 |
| 11 | 57 | Female | 153 | 70 | 29.90 | 0 | 608.53 | 0.24 | 680.13 | 2072.05 |
| 12 | 43 | Female | 150 | 80 | 35.56 | 0 | 548.45 | 0.21 | 645.00 | 2078.28 |
| 13 | 76 | Female | 150 | 36 | 16.00 | 1 | 518.41 | 0.30 | 635.44 | 2155.88 |
| 14 | 58 | Female | 163 | 45 | 16.94 | 1 | 472.87 | 0.22 | 563.38 | 2486.11 |
| 15 | 68 | Male | 160 | 49.5 | 19.34 | 1 | 844.96 | 0.18 | 679.47 | 2356.40 |
| 16 | 64 | Female | 168 | 56 | 19.84 | 1 | 588.18 | 0.25 | 632.64 | 2176.84 |
| 17 | 57 | Male | 168 | 57 | 20.20 | 1 | 435.08 | 0.25 | 575.74 | 2129.26 |
| 18 | 76 | Male | 175 | 62 | 20.24 | 1 | 422.48 | 0.31 | 573.11 | 2074.88 |
| 19 | 72 | Female | 150 | 46 | 20.44 | 1 | 427.33 | 0.33 | 562.22 | 2195.53 |
| 20 | 74 | Male | 170 | 60 | 20.76 | 1 | 672.48 | 0.19 | 652.01 | 2388.12 |
| 21 | 77 | Female | 148 | 48 | 21.91 | 1 | 482.56 | 0.23 | 679.63 | 2239.15 |
| 22 | 57 | Male | 158 | 55 | 22.03 | 1 | 582.36 | 0.33 | 629.50 | 2317.31 |
| 23 | 49 | Female | 160 | 57 | 22.27 | 1 | 636.63 | 0.32 | 704.53 | 2134.92 |
| 24 | 69 | Female | 155 | 54 | 22.48 | 1 | 689.92 | 0.24 | 675.51 | 2177.97 |
| 25 | 53 | Female | 162 | 60 | 22.86 | 1 | 393.41 | 0.26 | 635.27 | 2604.49 |
| 26 | 55 | Female | 168 | 65 | 23.03 | 1 | 416.67 | 0.25 | 704.20 | 2405.68 |
| 27 | 66 | Female | 167 | 70 | 25.10 | 1 | 395.35 | 0.29 | 610.87 | 2569.38 |
| 28 | 58 | Female | 172 | 75 | 25.35 | 1 | 594.96 | 0.34 | 722.67 | 2181.94 |
| 29 | 71 | Female | 160 | 65 | 25.39 | 1 | 434.11 | 0.28 | 562.88 | 2139.45 |
| 30 | 70 | Female | 150 | 60.5 | 26.89 | 1 | 728.68 | 0.20 | 575.41 | 2529.73 |
| 31 | 64 | Female | 158 | 69 | 27.64 | 1 | 511.63 | 0.27 | 722.18 | 2325.24 |
| 32 | 64 | Male | 170 | 80 | 27.68 | 1 | 730.62 | 0.31 | 724.98 | 2078.28 |
| 33 | 42 | Female | 169 | 80 | 28.01 | 1 | 672.48 | 0.32 | 584.98 | 2235.75 |
| 34 | 67 | Female | 158 | 70 | 28.04 | 1 | 477.71 | 0.26 | 603.94 | 2360.36 |
| 35 | 62 | Female | 163 | 75 | 28.23 | 1 | 570.74 | 0.17 | 573.76 | 2132.09 |
| 36 | 52 | Male | 178 | 97 | 30.61 | 1 | 738.37 | 0.32 | 722.18 | 2365.46 |
| 37 | 54 | Female | 155 | 75 | 31.22 | 1 | 760.66 | 0.26 | 588.77 | 2074.88 |
| 38 | 52 | Female | 165 | 85 | 31.22 | 1 | 720.72 | 0.27 | 713.44 | 2193.83 |
| 39 | 63 | Female | 158 | 85 | 34.05 | 1 | 900.90 | 0.31 | 571.29 | 2435.13 |
| 40 | 53 | Female | 156 | 85 | 34.93 | 1 | 559.11 | 0.22 | 602.38 | 2163.81 |
| 41 | 59 | Female | 164 | 57 | 21.19 | 2 | 409.88 | 0.26 | 609.30 | 2189.30 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 42 | 60 | Female | 160 | 55 | 21.48 | 2 | 641.64 | 0.24 | 605.26 | 2601.66 |
| 43 | 65 | Male | 173 | 65 | 21.72 | 2 | 697.18 | 0.23 | 686.56 | 2278.80 |
| 44 | 66 | Female | 155 | 54 | 22.48 | 2 | 736.43 | 0.24 | 634.61 | 2403.98 |
| 45 | 66 | Female | 155 | 54 | 22.48 | 2 | 583.33 | 0.27 | 579.21 | 2096.41 |
| 46 | 75 | Female | 156 | 55 | 22.60 | 2 | 555.23 | 0.24 | 610.04 | 2156.45 |
| 47 | 60 | Female | 155 | 55 | 22.89 | 2 | 409.88 | 0.34 | 585.80 | 2588.63 |
| 48 | 50 | Male | 180 | 75 | 23.15 | 2 | 814.92 | 0.30 | 712.78 | 2273.70 |
| 49 | 75 | Female | 160 | 60 | 23.44 | 2 | 467.05 | 0.34 | 688.37 | 2030.13 |
| 50 | 70 | Female | 160 | 60 | 23.44 | 2 | 402.13 | 0.33 | 616.14 | 2190.43 |
| 51 | 57 | Female | 160 | 60 | 23.44 | 2 | 506.51 | 0.28 | 614.33 | 2349.03 |
| 52 | 70 | Female | 153 | 55 | 23.50 | 2 | 592.05 | 0.25 | 635.27 | 2456.09 |
| 53 | 54 | Male | 165 | 65 | 23.88 | 2 | 859.50 | 0.27 | 669.24 | 2078.85 |
| 54 | 47 | Female | 155 | 57.5 | 23.93 | 2 | 656.01 | 0.19 | 675.18 | 2236.88 |
| 55 | 64 | Female | 157 | 60 | 24.34 | 2 | 553.29 | 0.19 | 585.31 | 2244.24 |
| 56 | 72 | Female | 162 | 65 | 24.77 | 2 | 422.48 | 0.32 | 616.97 | 2275.40 |
| 57 | 75 | Female | 155 | 60 | 24.97 | 2 | 618.22 | 0.24 | 681.86 | 2238.30 |
| 58 | 69 | Female | 163 | 67 | 25.22 | 2 | 692.83 | 0.19 | 711.29 | 2607.33 |
| 59 | 69 | Female | 167 | 72 | 25.82 | 2 | 468.99 | 0.22 | 704.04 | 2085.08 |
| 60 | 68 | Male | 176 | 80 | 25.83 | 2 | 660.85 | 0.19 | 681.78 | 2076.58 |
| 61 | 70 | Female | 153 | 63 | 26.90 | 2 | 622.09 | 0.23 | 701.07 | 2621.49 |
| 62 | 63 | Female | 162 | 70.6 | 26.90 | 2 | 623.55 | 0.28 | 630.00 | 2479.88 |
| 63 | 68 | Female | 150 | 62 | 27.56 | 2 | 638.57 | 0.28 | 585.97 | 2319.58 |
| 64 | 69 | Female | 157 | 68 | 27.59 | 2 | 387.60 | 0.32 | 583.00 | 2191.57 |
| 65 | 50 | Female | 162 | 75 | 28.58 | 2 | 947.95 | 0.32 | 670.56 | 2290.12 |
| 66 | 73 | Female | 146 | 62 | 29.09 | 2 | 347.87 | 0.22 | 659.68 | 2385.85 |
| 67 | 56 | Female | 160 | 75 | 29.30 | 2 | 739.74 | 0.20 | 698.60 | 2531.42 |
| 68 | 64 | Female | 160 | 76 | 29.69 | 2 | 424.42 | 0.35 | 562.06 | 2275.40 |
| 69 | 58 | Female | 160 | 76.5 | 29.88 | 2 | 847.85 | 0.30 | 557.11 | 2282.19 |
| 70 | 71 | Female | 158 | 75 | 30.04 | 2 | 970.97 | 0.26 | 675.10 | 2110.28 |
| 71 | 68 | Male | 178 | 97 | 30.60 | 2 | 420.54 | 0.18 | 668.75 | 2442.49 |
| 72 | 61 | Female | 155 | 75 | 31.22 | 2 | 516.47 | 0.24 | 633.46 | 2452.57 |
| 73 | 62 | Male | 165 | 85 | 31.22 | 2 | 344.96 | 0.25 | 585.80 | 2274.26 |
| 74 | 74 | Male | 160 | 80 | 31.25 | 2 | 758.72 | 0.18 | 566.01 | 2317.31 |
| 75 | 57 | Female | 163 | 85 | 31.99 | 2 | 639.53 | 0.21 | 584.98 | 2286.60 |
| 76 | 51 | Male | 175 | 100 | 32.65 | 2 | 877.91 | 0.33 | 731.25 | 2171.20 |
| 77 | 72 | Female | 155 | 42 | 17.48 | 3 | 505.81 | 0.18 | 672.21 | 2164.38 |
| 78 | 58 | Male | 175 | 58 | 18.94 | 3 | 973.97 | 0.26 | 574.75 | 2596.00 |
| 79 | 66 | Male | 170 | 55 | 19.03 | 3 | 683.90 | 0.24 | 650.36 | 2335.44 |
| 80 | 66 | Female | 150 | 45 | 20.00 | 3 | 531.98 | 0.34 | 603.94 | 2494.61 |
| 81 | 67 | Male | 161 | 56.5 | 21.80 | 3 | 830.79 | 0.29 | 581.35 | 2255.57 |
| 82 | 65 | Female | 155 | 52.5 | 21.85 | 3 | 425.39 | 0.30 | 652.42 | 2366.59 |
| 83 | 85 | Female | 156 | 55 | 22.60 | 3 | 584.30 | 0.34 | 623.65 | 2275.96 |
| 84 | 69 | Female | 157 | 57 | 23.12 | 3 | 822.67 | 0.22 | 659.84 | 2272.57 |
| 85 | 58 | Female | 178 | 74 | 23.40 | 3 | 529.07 | 0.23 | 645.99 | 2129.56 |
| 86 | 74 | Malae | 165 | 64 | 23.51 | 3 | 827.55 | 0.24 | 625.21 | 2435.32 |
| 87 | 74 | Female | 149 | 53 | 23.87 | 3 | 674.42 | 0.27 | 641.05 | 2487.67 |
| 88 | 73 | Female | 165 | 65 | 23.88 | 3 | 847.87 | 0.20 | 596.69 | 2303.26 |

|  |  |  |  |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|
| 89 | 73 | Female | 165 | 67.5 | 24.79 | 3 | 626.45 | 0.31 | 652.51 | 2248.24 |
| 90 | 74 | Female | 163 | 67 | 25.22 | 3 | 562.02 | 0.21 | 610.21 | 2185.48 |
| 91 | 72 | Female | 160 | 65 | 25.39 | 3 | 500.00 | 0.34 | 694.80 | 2122.42 |
| 92 | 66 | Female | 160 | 66.2 | 25.90 | 3 | 766.47 | 0.20 | 605.92 | 2488.86 |
| 93 | 61 | Female | 158 | 68 | 27.24 | 3 | 669.57 | 0.21 | 571.95 | 2487.07 |
| 94 | 78 | Female | 154 | 65 | 27.41 | 3 | 626.94 | 0.31 | 601.63 | 2487.07 |
| 95 | 74 | Female | 164 | 74 | 27.50 | 3 | 566.86 | 0.25 | 625.21 | 2121.83 |
| 96 | 75 | Male | 176 | 86 | 27.76 | 3 | 524.22 | 0.31 | 657.54 | 2124.21 |
| 97 | 58 | Female | 160 | 72 | 28.13 | 3 | 905.91 | 0.32 | 650.61 | 2124.21 |
| 98 | 64 | Female | 164 | 78 | 29.00 | 3 | 843.02 | 0.34 | 624.39 | 2168.23 |
| 99 | 58 | Female | 165 | 79 | 29.02 | 3 | 713.18 | 0.26 | 642.53 | 2171.20 |
| 100 | 75 | Male | 173 | 87 | 29.07 | 3 | 721.90 | 0.24 | 719.04 | 2196.19 |
| 101 | 76 | Male | 165 | 80 | 29.38 | 3 | 1046.05 | 0.23 | 614.33 | 2168.82 |
| 102 | 79 | Male | 182 | 100 | 30.19 | 3 | 584.58 | 0.31 | 720.86 | 2597.12 |
| 103 | 65 | Male | 170 | 87.5 | 30.28 | 3 | 796.80 | 0.32 | 562.88 | 2196.19 |
| 104 | 63 | Female | 156 | 77.5 | 31.80 | 3 | 554.26 | 0.30 | 695.63 | 2168.23 |
| 105 | 50 | Female | 153 | 75 | 32.04 | 3 | 1100.10 | 0.33 | 596.52 | 2663.15 |
| 106 | 62 | Female | 160 | 85 | 33.20 | 3 | 505.81 | 0.24 | 591.08 | 2475.18 |
| 107 | 55 | Male | 170 | 62 | 21.45 | 4 | 894.89 | 0.26 | 610.54 | 2347.28 |
| 108 | 54 | Female | 150 | 52 | 23.11 | 4 | 784.88 | 0.22 | 711.46 | 2149.79 |
| 109 | 69 | Male | 178 | 74 | 23.40 | 4 | 404.07 | 0.34 | 731.74 | 2347.28 |
| 110 | 83 | Female | 165 | 65 | 23.88 | 4 | 804.26 | 0.23 | 647.81 | 2470.42 |
| 111 | 65 | Female | 163 | 65 | 24.46 | 4 | 600.78 | 0.23 | 683.67 | 2410.04 |
| 112 | 75 | Male | 169 | 74 | 25.91 | 4 | 557.17 | 0.34 | 595.70 | 2169.42 |
| 113 | 77 | Male | 161 | 71 | 27.39 | 4 | 631.78 | 0.26 | 567.50 | 2458.52 |
| 114 | 65 | Male | 175 | 86 | 28.08 | 4 | 642.44 | 0.25 | 587.12 | 2390.71 |
| 115 | 70 | Female | 156 | 66 | 28.12 | 4 | 860.47 | 0.34 | 689.53 | 2274.11 |
| 116 | 70 | Male | 173 | 87 | 29.07 | 4 | 721.90 | 0.30 | 612.52 | 2149.19 |
| 117 | 71 | Female | 158 | 74 | 29.64 | 4 | 875.00 | 0.31 | 673.12 | 2332.71 |
| 118 | 76 | Male | 165 | 73 | 31.25 | 4 | 715.12 | 0.30 | 696.95 | 2205.70 |
| 119 | 52 | Female | 155 | 80 | 33.30 | 4 | 1137.14 | 0.32 | 579.21 | 2607.83 |
| 120 | 63 | Female | 160 | 89 | 34.77 | 4 | 675.39 | 0.22 | 624.72 | 2588.79 |

BMI= Body Mass Index; K & L Grade=Kellgren & Lawrence Grade; FFA=free fatty acids; TG=triglyceride; FC=free cholesterol; TC= total cholesterol

**Supplementary Table 3. Oligonucleotide and primer list.**

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| **si/shRNA for gene knockdown** | | |
| *Hadha* si/shRNA #1: CCAATACAGAATAGCAACATT | This paper | N/A |
| *Hadha* si/shRNA #2: GGGATTCCATCTTCAGCAATT | This paper | N/A |
| *Hadhb* si/shRNA #1: GTACAGTTATTCAGGAAGTTT | This paper | N/A |
| *Hadhb* si/shRNA #2: CTGTCACCATGGCTTGTATTT | This paper | N/A |
| *TRIM9* si/shRNA #1: GACAAGATGAGCCTATACATT | This paper | N/A |
| *TRIM9* si/shRNA #2: GACCTGTAGTAGCTATGATTT | This paper | N/A |
| **gRNA for gene knockout** | | |
| *Hadha* sgRNA: AAGTCCACAAAGCCTATTG | This paper | N/A |
| *Hadhb* sgRNA: TACCTGGGGCAGCTCGTAGC | This paper | N/A |
| **Primers for RT-qPCR** | | |
| *β-actin*-F: GGCTGTATTCCCCTCCATCG | This paper | N/A |
| *β-actin*-R: CCAGTTGGTAACAATGCCATGT | This paper | N/A |
| *Col2a1*-F: TGGCTTAGGGCAGAGAGAGA | This paper | N/A |
| *Col2a1*-R: CGTCGTGCTGTCTCAAGGT | This paper | N/A |
| *Acan*-F: CCTGCTACTTCATCGACCCC | This paper | N/A |
| *Acan*-R: AGATGCTGTTGACTCGAACCT | This paper | N/A |
| *Sox9*-F: TAATTCCCCAGGCTCTTGGAT | This paper | N/A |
| *Sox9*-R: GCAGCCGGGATTTAAGGCTC | This paper | N/A |
| *Sox5*-F: CCCGTGATCCAGAGCACTTAC | This paper | N/A |
| *Sox5*-R: CCGCAATGTGGTTTTCGCT | This paper | N/A |
| *Sox6*-F: GGTCATGTTTCCCACCCACAA | This paper | N/A |
| *Sox6*-R: TTCAGAGGGGTCCAAATTCCT | This paper | N/A |
| *Hdac3*-F: GCCAAGACCGTGGCGTATT | This paper | N/A |
| *Hdac3*-R: GTCCAGCTCCATAGTGGAAGT | This paper | N/A |
| *Pdk4*-F: AGGGAGGTCGAGCTGTTCTC | This paper | N/A |
| *Pdk4*-R: GGAGTGTTCACTAAGCGGTCA | This paper | N/A |
| *Mmp13*-F: CAAGCAGTTCCAAAGGCTACA | This paper | N/A |
| *Mmp13*-R: TAGGGCTGGGTCACACTTCT | This paper | N/A |
| *Adamts7*-F: AGTGTCCCAACCTGCCATTTG | This paper | N/A |
| *Adamts7*-R: CCTAGAGCCTTGGTGCTTGTA | This paper | N/A |
| *Lipe*-F: CCAGCCTGAGGGCTTACTG | This paper | N/A |
| *Lipe*-R: CTCCATTGACTGTGACATCTCG | This paper | N/A |
| *Cd36*-F: GGAGCCATCTTTGAGCCTTCA | This paper | N/A |
| *Cd36*-R: GAACCAAACTGAGGAATGGATCT | This paper | N/A |
| *Fatp5*-F: GTTCTCCCGTCCAAGACCATT | This paper | N/A |
| *Fatp5*-R: GCTCCGTACAGAGTGTAGCAAG | This paper | N/A |
| *Fabp3*-F: ACCTGGAAGCTAGTGGACAG | This paper | N/A |
| *Fabp3*-R: TGATGGTAGTAGGCTTGGTCAT | This paper | N/A |
| *Apob*-F: GAGACAGGAGCAGTAATACAGCC | This paper | N/A |
| *Apob*-R: GGCCACCCAGTCTATCTCTTTG | This paper | N/A |
| *Srebf1*-F: GATGTGCGAACTGGACACAG | This paper | N/A |
| *Srebf1*-R: CATAGGGGGCGTCAAACAG | This paper | N/A |
| *Srebf2*-F: GCAGCAACGGGACCATTCT | This paper | N/A |
| *Srebf2*-R: CCCCATGACTAAGTCCTTCAACT | This paper | N/A |

| | | |
|---|---|---|
| *Esr1*-F: CCTCCCGCCTTCTACAGGT | This paper | N/A |
| *Esr1*-R: CACACGGCACAGTAGCGAG | This paper | N/A |
| **Primers for CUT&Tag-qPCR** | | |
| DNA Spike-in-F: GCCTTCTTCCCATTTCTGATCC | This paper | N/A |
| DNA Spike-in-R: CACGAATCAGCGGTAAAGGT | This paper | N/A |
| *Col2a1*-peak10126-F: ACTGAGCTCGAAACGTCCTG | This paper | N/A |
| *Col2a1*-peak10126-R: GACCTCTGTCGCAGCATCTT | This paper | N/A |
| *Col2a1*-peak10129-F: TCAGATAGAGCACCTGGCCT | This paper | N/A |
| *Col2a1*-peak10129-R: AAAGGCCTCTGATGCTTCCC | This paper | N/A |
| *Acan*-peak24681-F: CAGTGGCTCTCCAGCTTCTC | This paper | N/A |
| *Acan*-peak24681-R: CTCCTTCTCCTCTCTGCCCT | This paper | N/A |
| *Acan* peak24683-F: ATTCCCAGAGTAGGGGGTCG | This paper | N/A |
| *Acan*-peak24683-R: GGGTGTTTCCCTGGTATCGG | This paper | N/A |
| *Sox5*-peak23700-F: CCAGGTGCGGTGTCATATCT | This paper | N/A |
| *Sox5*-peak23700-R: GAGGTGGTTCAAACAGCCGA | This paper | N/A |
| *Sox5*-peak23705-F: TGTGACAGTCAGCTCGTCTC | This paper | N/A |
| *Sox5*-peak23705-R: GGATCGGTTCCAAGCCCAA | This paper | N/A |
| *Sox6*-peak25230-F: CAGAGGAGGCCAGCATTTGA | This paper | N/A |
| *Sox6*-peak25230-R: TTCCGTGGACATGAGAAGCC | This paper | N/A |
| *Sox6*-peak25234-F: AACCCCCTGTCTATCGCTGT | This paper | N/A |
| *Sox6*-peak25234-R: AGTCTGCTGGCTGTGAAGAT | This paper | N/A |
| *Pdk4*-peak22212-F: GCAAGGACCACCCTACAGTT | This paper | N/A |
| *Pdk4*-peak22212-R: ACCCTCAGCAAATCGCTCTT | This paper | N/A |
| *Mmp13*-peak27329-F: GAAGCTCAAGGATGCGCCTA | This paper | N/A |
| *Mmp13*-peak27329-R: AACCAGTTGGCCTCAGTGTT | This paper | N/A |
| *Adamts7*-peak28458-F: AGCAACTTCTGCTCTGCCAT | This paper | N/A |
| *Adamts7*-peak28458-R: GTGCTGAGTGTCTGTGGGAA | This paper | N/A |
| **Primers for mice identification** | | |
| Acan-CreER$^{T2}$-F WT: GTTATATTCCGGAGCCCACA | This paper | N/A |
| Acan-CreER$^{T2}$-F Mut: CTCCAGACTGCCCTTGGGAAAA | | |
| Acan-CreER$^{T2}$-R Common: AAAAGCGACAAGAAGACACCA | This paper | N/A |
| Col2-CreER$^{T2}$-F: GAACGCACTGATTTCGACCA | This paper | N/A |
| Col2-CreER$^{T2}$-R: GCTAACCAGCGTTTTCGTTC | This paper | N/A |
| *Hadha*$^{fl/fl}$-F: GTGAAGAAACCACCTGACATTGG | This paper | N/A |
| *Hadha*$^{fl/fl}$-R: ACTGGTCCTTATGAATGGAGTAGG | This paper | N/A |
| *Hadhb*$^{fl/fl}$-F: CTAAATTATCCCACCACGACCC | This paper | N/A |
| *Hadhb*$^{fl/fl}$-R: CCAGAGGACAGTGCTCATTTAGTA | This paper | N/A |
| *Hadha*$^{CKI/CKI}$ -Mut-F: TCAGACTGGTCCGAATCCAC | This paper | N/A |
| *Hadha*$^{CKI/CKI}$-Mut-R: TGCACAAGGATAAAGGAAGGCAAC | This paper | N/A |
| *Hadha*$^{CKI/CKI}$ -WT-F: CGAGGTGTAAGTTGATTTGAGTGA | This paper | N/A |
| *Hadha*$^{CKI/CKI}$-WT-R: TGCACAAGGATAAAGGAAGGCAAC | This paper | N/A |

**Supplementary Table 4. The OA cartilage histopathology grade.**

| Grade (key feature) | Associated criteria (tissue reaction) |
|---|---|
| Grade 0: surface intact, cartilage morphology intact | Matrix: normal architecture |
| | Cells: intact, appropriate orientation |
| Grade 1: surface intact | Matrix: superficial zone intact, oedema and/or superficial fibrillation (abrasion), focal superficial matrix condensation |
| | Cells: death, proliferation (clusters), hypertrophy, superficial zone |
| | Reaction must be more than superficial fibrillation only |
| Grade 2: surface discontinuity | As above |
| | +Matrix discontinuity at superficial zone (deep fibrillation) |
| | ±Cationic stain matrix depletion (Safranin O or Toluidine Blue) upper 1/3 of cartilage |
| | ±Focal perichondronal increased stain (mid zone) |
| | ±Disorientation of chondron columns |
| | Cells: death, proliferation (clusters), hypertrophy |
| Grade 3: vertical fissures (clefts) | As above |
| | Matrix vertical fissures into mid zone, branched fissures |
| | ±Cationic stain depletion (Safranin O or Toluidine Blue) into lower 2/3 of cartilage (deep zone) |
| | ± New collagen formation (polarized light microscopy, Picro Sirius Red stain) |
| | Cells: death, regeneration (clusters), hypertrophy, cartilage domains adjacent to fissures |
| Grade 4: erosion | Cartilage matrix loss: delamination of superficial layer, mid layer cyst formation |
| | Excavation: matrix loss superficial layer and mid zone |
| Grade 5: denudation | Surface: sclerotic bone or reparative tissue including fibrocartilage within denuded surface. Microfracture with repair limited to bone surface |
| Grade 6: deformation | Bone remodelling (more than osteophyte formation only). Includes: microfracture with fibrocartilaginous and osseous repair extending above the previous surface |

**Supplementary Table 5. The recommended semi-quantitative scoring system (OARSI score).**

| **Grade** | **Osteoarthritic damage** |
|---|---|
| 0 | Normal |
| 0.5 | Loss of Safranin-O without structural changes |
| 1 | Small fibrillations without loss of cartilage |
| 2 | Vertical clefts down to the layer immediately below the superficial layer and some loss of surface lamina |
| 3 | Verical clefts/erosion to the calcified cartilage extending to <25% of the articular surface |
| 4 | Vertical clefts/erosion to the calcified cartilage extending to 25–50% of the articular surface |
| 5 | Vertical clefts/erosion to the calcified cartilage extending to 50–75% of the articular surface |
| 6 | Vertical clefts/erosion to the calcified cartilage extending >75% of the articular surface |

**Supplementary Table 6. The Mankin Histology histopathology grading system.**

| | **Grade** |
|---|---|
| **I. Structure** | |
| a. Normal | 0 |
| b. Surface irregularities | 1 |
| c. Pannus and surface irregularities | 2 |
| d. Clefts to transitional zone | 3 |
| e. Clefts to radial zone | 4 |
| f. Clefts to calcified zone | 5 |
| g. Complete disorganisation | 6 |
| **II. Cells** | |
| a. Normal | 0 |
| b. Diffuse hypercellularity | 1 |
| c. Cloning | 2 |
| d. Hypocellularity | 3 |
| **III. Safranin-O staining** | |
| a. Normal | 1 |
| b. Slight reduction | 2 |
| c. Moderate reduction | 3 |
| d. Severe reduction | 4 |
| e. No dye noted | 5 |
| **IV. Tidemark integrity** | |
| a. Intact | 0 |
| b. Crossed by blood vessels | 1 |

Mankin HHGS score is the sum of structure, cells, Safranin-O staining and tidemark integrity.

**Supplementary Table 7. Multiple reaction monitoring parameters**

| Compound | Precursor (m/z) | Product(m/z) | CE(V) | RF Lens (V) | Polarity | IS |
|---|---|---|---|---|---|---|
| Acetyl-CoA | 810 | 303.05 | 31.14 | 102 | + | 4-CPA |
| $^{13}C_2$-Acetyl-CoA | 812 | 305.05 | 30.49 | 143 | + | 4-CPA |
| 4-CPA | 200 | 154 | 13 | 59 | + | |

**Supplementary Table 8. Key resources table.**

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| Antibodies | | |
| Rabbit monoclonal Anti- HADHA | Abcam | Cat#ab203114 |
| Rabbit polyclonal Anti-HADHB | Abcam | Cat#ab230667 |
| Rabbit monoclonal Anti-SOX9 | Abcam | Cat#ab185230 |
| Rabbit monoclonal Anti-MMP3 | Abcam | Cat#ab52915 |
| Rabbit polyclonal Anti-MMP13 | Abcam | Cat#ab39012 |
| Rabbit polyclonal Anti- Acetyl Lysine | Abcam | Cat#ab21623 |
| Rabbit monoclonal Anti-Ubiquitin | Abcam | Cat#ab134953 |
| Rabbit monoclonal Anti-TSG101 | Abcam | Cat#ab125011 |
| Rabbit monoclonal Anti-Phospho-ACC (Ser79) | Cell Signaling Technology | Cat# 11818 |
| Rabbit monoclonal Anti-ACC | Cell Signaling Technology | Cat #3676 |
| Rabbit monoclonal Anti-Phospho-AMPKα (Thr172) | Cell Signaling Technology | Cat# 2531 |
| Rabbit monoclonal Anti-AMPKα | Cell Signaling Technology | Cat#5831 |
| Rabbit monoclonal Anti- Phospho-AMPK Substrate Motif | Cell Signaling Technology | Cat# 5759 |
| Mouse monoclonal Anti-Beta Actin | Proteintech | Cat#66009-1-Ig |
| Mouse monoclonal Anti-GAPDH | Proteintech | Cat#60004-1-Ig |
| Mouse monoclonal Anti-CD63 | Proteintech | Cat#67605-1-Ig |
| Rabbit polyclonal Anti-TRIM9 | Proteintech | Cat#10786-1-AP |
| Rabbit polyclonal anti-PDK4 | Proteintech | Cat#12949-1-AP |
| Mouse monoclonal anti-ACAN | Millipore | Cat#C8035 |
| Rabbit polyclonal Anti-Acetyl-HADHA at Lysine 728 | this paper | N/A |
| Mouse Monoclonal Anti-Histone H3 | Beyotime Biotechnology | Cat#Cat: AF0009 |
| Rabbit polyclonal Anti-Histone H3K27ac | Active Motif | Cat#39133 |
| Rabbit polyclonal Anti-COL2A1 | Bioss | Cat#bs-10589R |
| Mouse monoclonal Anti-FLAG HRP | Sigma-Aldrich | Cat#A8592 |
| Mouse monoclonal Anti-HA HRP | GenScript | Cat#A01296 |
| Anti-DYKDDDDK IP Resin | GenScript | Cat#L00425 |
| Anti-HA magnetic beads | Selleck chemicals | Cat#B26202 |
| Goat Anti-Mouse HRP | FUDE Biotech | Cat#FDM007 |
| Goat Anti-Rabbit HRP | FUDE Biotech | Cat#FDR007 |
| Alexa Fluor 594 goat anti-mouse IgG | FUDE Biotech | Cat#FD0143 |
| Alexa Fluor 488 goat anti-rabbit IgG | FUDE Biotech | Cat#FD0136 |
| Biological Samples | | |
| Human cartilage samples, see Table S1 | Sir Run Run Shaw Hospital,<br>Zhejiang University School of Medicine | N/A |
| Human synovial fluid, see Table S2 | Sir Run Run Shaw Hospital,<br>Zhejiang University School of Medicine | N/A |

| | | |
|---|---|---|
| Healthy adult peripheral blood | Volunteers from Sir Run Run Shaw Hospital, Zhejiang University School of Medicine | N/A |
| Chemicals, Peptides, and Recombinant Proteins | | |
| IBMX | Sigma-Aldrich | Cat#I7018 |
| Palmitic acid | Sigma-Aldrich | Cat#P0500 |
| Oleic acid | Sigma-Aldrich | Cat#O1008 |
| Acetyl-1,2-$^{13}\mathrm{C}_2$ coenzyme A lithium salt | Sigma-Aldrich | Cat#658650 |
| DL-β-Hydroxybutyryl coenzyme A lithium salt | Sigma-Aldrich | Cat#H0261 |
| NAD | Sigma-Aldrich | Cat#NAD98-RO |
| 1-MPMS | Sigma-Aldrich | Cat#M8640 |
| FCCP | TargetMOI | Cat#T6834 |
| Oligomycin A | TargetMOI | Cat#T6323 |
| Rotenone | TargetMOI | Cat#T2970 |
| Trichostatin A | TargetMOI | Cat#T6270 |
| A-769662 | TargetMOI | Cat#T2468 |
| Dorsomorphin dihydrochloride | TargetMOI | Cat#T6146 |
| Cycloheximide (CHX) | Selleck chemicals | Cat#S7418 |
| Etomoxir sodium salt | Selleck chemicals | Cat#S8244 |
| Trimetazidine dihydrochloride | Selleck chemicals | Cat#S4543 |
| MG132 | Selleck chemicals | Cat#S2619 |
| Chloroquine | Selleck chemicals | Cat#S6999 |
| Recombinant Human Insulin | Solarbio | Cat#I8830 |
| Dexamethasone | Solarbio | Cat#D8040 |
| Nile red | MCE | Cat#HY-D0718 |
| BODIPY 493/503 | MCE | Cat#HY-D1614 |
| WST-8 | MCE | Cat#HY-D0831 |
| UK5099 | MCE | Cat#HY-15475 |
| BPTES | MCE | Cat#HY-12683 |
| Ca2+ ionophore | Abcam | Cat#ab120370 |
| Antimycin A | Abcam | Cat#ab141904 |
| Nicotinamide | Sangon Biotech | Cat#A510659 |
| Recombinant Human TNF-alpha | R&D systems | Cat#10291-TA |
| Recombinant Human IL-1 beta | R&D systems | Cat#201-LB |
| Fatty Acid Mix (U-13C, 98%) | Cambridge Isotope Laboratories | Cat#CLM-8455-PK |
| 3×FALG peptide | YEASEN | Cat#20571ES |
| SYBR Green Master Mix | Vazyme Biotech | Cat#Q711-02 |
| TRIzol | Takara | Cat#9109 |
| Collagenase II | Sigma-Aldrich | Cat#C2-BIOC |
| Antifade mounting medium with DAPI | Meilunbio | Cat#MA0222 |
| FBS | Cellmax | Cat#SA101.02 |
| NBCS | Gibco | Cat#16010159 |

| | | |
|---|---|---|
| Critical Commercial Assays | | |
| Free Fatty Acid Uptake Assay | Abcam | Cat#ab176768 |
| Acetyl-CoA ELISA Kit | Elabscience | Cat#E-EL-0125c |
| ATP Chemiluminescence Assay Kit | Elabscience | Cat#E-BC-F002 |
| Seahorse XFe96 FluxPaks | Agilent | Cat#103793-100 |
| Seahorse XF Palmitate-BSA FAO Substrate | Agilent | Cat#102720-100 |
| Nonesterified Free fatty acids (NEFA) assay kit | Aidisheng Biotech | Cat#DS-W-ZF001-96 |
| Triglyceride (TG) Assay Kit | Aidisheng Biotech | Cat#ADS-W-ZF013 |
| Total Cholesterol Assay Kit | Meimian Biotech | Cat#MM-50977H1 |
| Free Cholesterol Assay Kit | Meimian Biotech | Cat#MM-63462H1 |
| Hyperactive Universal CUT&Tag Assay Kit for Illumina Pro | Vazyme Biotech | Cat#TD904-02 |
| Mouse Direct PCR kit | Bimake | Cat# B40015 |
| Super Vision IHC kit | BOSTER | Cat#SV0004 |
| BCA protein assay kit | Meilunbio | Cat#MA0082 |
| Special deparaffinized Reagent Kit for Lipid Staining | HePeng Biotech | Cat#HPBIO-JM4801 |
| SteadyPure RNA extraction kit | Accurate Biotechnology | Cat#AG21017 |
| Experimental Models: Cell Lines | | |
| C28/I2 | Sigma-Aldrich | Cat#SCC043 |
| 3T3-L1 | ATCC | Cat#CL-173 |
| HEK293T | ATCC | Cat#CRL-11268 |
| HADHA-KO C28/I2 | this paper | N/A |
| HADHB-KO C28/I2 | this paper | N/A |
| Primary mouse chondrocytes | this paper | N/A |
| Experimental Models: Organisms/Strains | | |
| Mouse: C57BL/6 | Hangzhou QiZhen Laboratory Animal Technology Center | N/A |
| Mouse: Acan-CreER$^{T2}$ | Prof. Ximei Wu from Zhejiang University School of Medicine | N/A |
| Mouse: Col2-CreER$^{T2}$ | Jackson Laboratory | N/A |
| *Hadha*$^{fl/fl}$ | Cyagen Biology Technology | N/A |
| *Hadhb*$^{fl/fl}$ | Cyagen Biology Technology | N/A |
| *Hadha*$^{CKI/CKI}$ (p. K728R Conditional Knock in) | Cyagen Biology Technology | N/A |
| Oligonucleotides | | |
| si/shRNA for gene knockdown, see Table S3 | this paper | N/A |
| Primers for RT-qPCR, see Table S3 | this paper | N/A |
| Primers for CUT&Tag-qPCR, see Table S3 | this paper | N/A |
| gRNA for gene knockout, see Table S3 | this paper | N/A |
| Primers for mice identification, see Table S3 | this paper | N/A |
| Recombinant DNA | | |
| PLKO.1 puro | Addgene | Cat#8453 |

| | | |
|---|---|---|
| PEP-KO | this paper | N/A |
| pLVX-HADHA-Flag | this paper | N/A |
| pLVX-HADHA K728R-Flag | this paper | N/A |
| pLVX-HADHB-Flag | this paper | N/A |
| pLVX -HADHA-HA | this paper | N/A |
| pcDNA3.1-SOX9-Flag | this paper | N/A |
| pcDNA3.1-SOX9 T25A-Flag | this paper | N/A |
| pcDNA3.1-SOX9 T87A-Flag | this paper | N/A |
| pcDNA3.1-SOX9 S98A-Flag | this paper | N/A |
| pcDNA3.1-SOX9 S136A-Flag | this paper | N/A |
| pcDNA3.1-SOX9 S138A-Flag | this paper | N/A |
| pcDNA3.1-SOX9 T193A-Flag | this paper | N/A |
| pcDNA3.1-SOX9 ST-A-Flag (T25A, T87A, S98A, S136A, S138A, and T193A) | this paper | N/A |
| pcDNA3.1-SOX9 ST-D-Flag (T25D, T87D, S98D, S136D, S138D, and T193D) | this paper | N/A |
| pLVX-HA-ZNF598 | this paper | N/A |
| pLVX-HA-TRIM9 | this paper | N/A |
| pLVX-HA-RLIM | this paper | N/A |
| Software and Algorithms | | |
| GraphPad Prism 9.2.1 | https://www.graphpad.com/ | N/A |
| R 4.1.2 | https://www.r-project.org/ | N/A |
| Image J v1.50 | https://imagej.net/ | N/A |
| Wave 2.4.0 | https://www.agilent.com/ | N/A |
| IGV_2.16.2 | https://igv.org/ | N/A |
| NIS-Elements Viewer 4.20 | https://www.microscope.healthcare.nikon.com/ | N/A |
| Other | | |
| Safranin O | Sigma-Aldrich | Cat#S8884 |
| Fast Green | Sigma-Aldrich | Cat#F7252 |
| Tamoxifen | Sigma-Aldrich | Cat#T2859 |
| Alcian Blue 8 GS | Solarbio | Cat#IA2460 |
| Nuclear fast red | Solarbio | Cat# G1321 |
| Neutral balsam | Solarbio | Cat#G8590 |
| Protease Inhibitor Cocktail | TargetMOI | Cat#C0001 |
| PMSF | FUDE Biotech | Cat#FD0100 |
| Bovine Serum Albumin (fatty acids free) | Meilunbio | Cat#MB0094-1 |
| HFD with 60% of the calories from fat | Research Diets | Cat#D12492 |
| Normal diet | XIETONG BIO-ENGINEERING CO., LTD. | Cat#XTCON50J |
| Polybrene | Tsingke | Cat#40804ES76 |
| Protein A/G magnetic beads | Thermo Fisher Scientific | Cat#88802 |
| Lipofectamine™ RNAiMAX | Thermo Fisher Scientific | Cat#13778030 |

| | | |
|---|---|---|
| Hieff Trans® Liposomal Transfection Reagent | YEASEN | Cat#40802ES03 |
| Hieff qPCR SYBR Green Master Mix | YEASEN | Cat#11201 |
| Evo MMLV RT Premix | Accurate Biotechnology | Cat#AG11706 |
| Penicillin-Streptomycin | Biosharp | Cat#BL505A |
| Amersham Protran 0.45 NC membranes | Cytiva | Cat#10600002 |
| 5 × Loading buffer | FUDE Biotech | Cat#FD002 |
| Dura ECL | FUDE Biotech | Cat#FD8020 |
| 3,3'-diaminobenzidine (DAB) | BOSTER | Cat#AR1027 |
| Hematoxylin | Beyotime | Cat#C0107 |
| Poly-D-lysine | Beyotime | Cat#ST508 |